STUDIES IN THE METABOLISM OF APPLES.

VI. PRELIMINARY INVESTIGATIONS ON THE RESPIRATION OF SLICED APPLE TISSUE.

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(Three Text-figures.)

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Introduction.

Previous papers of this series (Hackney, 1943, 1944) were concerned with the respiratory trends of Granny Smith apples at various stages of development and of maturity in store. It was shown that the causes underlying these trends were not the same at all stages of development and maturity. In order to continue the investigations it seemed advisable to develop a technique for the study of the respiratory behaviour of sliced apple tissue immersed in distilled water and other media. By supplying various respiratory inhibitors and possible respiratory substrates to the sliced tissue, information might be obtained about the nature and relative importance of the reactions involved in respiration at various stages of development and maturity. Similar methods have been applied to the study of the respiration of carrot tissue by Turner (1938) and others, of potato tissue by Boswell and Whiting (1938) and others, and of beet tissue by Bennet-Clark and Bexon (1943). Apple tissue presents peculiar difficulties not met with in work on other storage tissues, and the technique of other investigators has been modified accordingly. The present paper outlines this technique, and includes results of preliminary investigations on the respiration rates of tissues from various parts of the fruit.

Materials.

Most of the experiments were carried out on Granny Smith apples of the 1944 season; some experiments were carried out on fruits of the 1945 season. The apples used were part of the normal commercial (mid-April) picking from a selected orchard at Orange, New South Wales. They were placed in cool store (1°C.) within a few days of harvesting. Batches of fruit were removed from store as required and placed in a room maintained at 21°C. in 1944. Their subsequent behaviour was observed at this temperature. During 1945 the experimental temperature was 25°C.; facilities for keeping the fruits at the lower temperature were not available in 1945.

Preparation of Tissues.

In the preparation of the cut apple tissue, skin and flesh were always treated separately. The skin was sliced off very thinly, using a razor blade. The average thickness of the tissue classified as skin was about 0·15 mm. The strips of skin were cut into pieces about 6 mm. square. In the early experiments the cut skin was subsequently washed in aerated distilled water for about 20 minutes, and the surplus water was removed by carefully drying the tissue between filter papers. The skin was then divided into replicate sets, usually of 1 gm. fresh weight each. In the later experiments the tissue was placed in the respiration vessels without preliminary washing (see later section).

Discs of flesh were prepared in the following way: Cylinders of tissue were cut from the fruit by means of a sharp cork-borer 0·9 cm. in diameter. Portions obviously bruised were discarded, and the remainder of each cylinder was cut into slices of the desired thickness (usually 1 mm.) by means of a razor blade. It was shown, by experiments to be described in a later section, that the small variations in thickness, due to cutting the slices by hand, were not likely to cause inaccuracy in the measurement of respiration rate. In the early experiments the flesh discs were washed and dried in the same way as the skin slices. Replicate sets of flesh tissue were usually of 2 gm. fresh weight (weighed after washing). In later experiments the discs were not washed before being placed in the respiration vessels.

In experiments where a large number of replicate sets was required, tissue slices were cut from a number of similar apples and randomized during washing. There was no significant difference in respiratory behaviour between tissue sets taken from such randomized lots. In experiments where a small number of replicates was required, randomized sets of tissue from a single apple were used. Although there was considerable variability in respiration rate between tissue sets from different apples, there was good agreement between the respiration rates of tissue sets from the same apple.

It was not considered necessary to prepare the material under sterile conditions, as bacterial growth did not occur during the short duration of the experiments (cf., Turner, 1938).

The Warburg manometric technique was employed in the determination of the rates of oxygen uptake and carbon dioxide output of the tissue slices. Each of the Warburg vessels had two side-arms as well as a small inner receptacle within the central cavity (see Dixon, 1943, p. 49). Two gm. flesh tissue or 1 gm. skin were suspended in the experimental medium in the central cavity of the vessel.

In determinations of the rate of oxygen uptake, the carbon dioxide given off by the tissue was absorbed by means of a small piece of Whatman's No. 40 (starch-free) filter paper soaked with 0·3 c.c. of normal potassium hydroxide; absorption was found to be equally efficient whether the filter paper was placed in the inner receptacle or in one of the side-arms. The vessels were immersed in a water-bath maintained at the experimental temperature (21°C. in 1944, 25°C. in 1945), and shaken at approximately 110 complete oscillations per minute.

In experiments where it was necessary to determine the respiration rate of the whole apple before cutting, the Pettenkofer method was adopted. These experiments were carried out in a room maintained at the desired temperature.

RESPIRATORY BEHAVIOUR AFTER CUTTING.

It is well known that the damaging of living tissue due to wounding frequently brings about an increase in the respiration rate. This increase may be due to a variety of causes.

Turner (1940) found that carrot tissue must be washed in aerated distilled water for 200 hours or more in order to minimize the preliminary period of abnormally high respiration. It was not practicable to follow this procedure with apple tissue, as it lost nearly all its respiratory activity when washed for periods longer than about 30 hours. Flesh tissue washed in aerated phosphate buffer (pH 5·9) did not survive longer than similar tissue in distilled water.

In the early work apple tissue was washed for 20 minutes (see later section) and suspended in distilled water for the duration of the experiments. The respiration rate was approximately the same whether the volume of water used was 1 c.c., 3 c.c., or 5 c.c. per gm. of flesh. For purposes of convenience 4·5 c.c. was the volume generally used.

Shape of the Respiration-Time Curve.—Although variations frequently occurred in the magnitude of the respiration rate, the shape of the respiration-time curve for flesh discs in distilled water was always the same at the experimental temperatures considered. Soon after cutting, the rates of oxygen uptake and carbon dioxide output were comparatively high (23–30 $mm.^3$/gm. initial wt./hr.). During the first 2 or 3 hours after cutting, the respiration rate decreased, subsequently reaching an almost steady level, which was maintained with very little change during the remainder of the period of observation (a further 4–6 hours). A typical curve for oxygen uptake of flesh tissue is shown in Figure 1. The shape of the respiration-time curve and the magnitude of the respiration rate were the same in phosphate buffer as in distilled water. The shape of the respiration-time curve is discussed further in a later paragraph.

The respiration-time curve for skin did not show the preliminary fall; it either remained approximately steady or rose slightly during the period of observation.

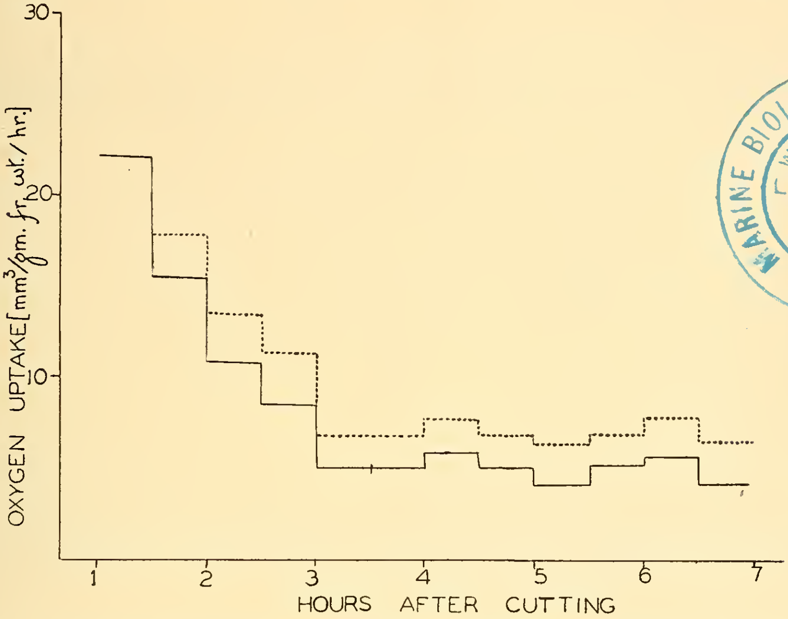

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Fig. 1.—Typical respiration-time curve for flesh of Granny Smith apple at 21°C. Time of preliminary washing was 20 minutes. The entire lines denote respiration rate based on the weight of the sample at the time of setting up in the Warburg vessel; the dotted lines denote respiration rate corrected to the true weight of the sample at the time of each observation (see section on effect of immersion in distilled water).

Effect of Immersion of Tissue in Distilled Water.—It was not until many investigations had been carried out using tissue washed in distilled water that it was discovered that considerable losses occurred in both fresh and dry weights when apple flesh was surrounded by distilled water. This behaviour was surprising, as no comparable loss in weight occurs in carrot, potato or beet tissue.

In order to determine the extent of loss in dry weight during washing, samples of 2 gm. fresh weight each were taken from a large batch of flesh discs after various periods of washing. The dry weights of these samples were determined. It was found that the ratio dry wt./fresh wt. decreased as the duration of the washing period increased (see Table 1).

In a second experiment the changes in fresh weight of a single sample of flesh discs were observed after various periods of washing. It was found that fresh weight decreased considerably as the duration of the period of washing increased (see Table 2). The dry weight of the sample after each period of washing was calculated from the ratios dry wt./fresh wt. in Table 1.

From these and similar experiments it was evident that during 2 hours in distilled water the apple flesh lost considerable proportions of both fresh and dry weight. The rates of loss were greatest during the first 20 minutes of washing and decreased as the length of the washing period increased.

TABLE 1.
Changes in Dry Wt./Fresh Wt. of Flesh Tissue after Various Periods of Washing in Distilled Water.

Period of Washing.	Fresh Wt. (gm.).	Dry Wt. (gm.).	Dry Wt./Fresh Wt. (%).
0	2	0·289	14·45
5 min.	,,	0·219	10·95
20 ,,	,,	0·180	9·00
60 ,,	,,	0·146	7·40
120 ,,	,,	0·118	6·50

Unlike the flesh, the skin showed an increase (25%) in fresh weight due to uptake of water during the first 20 minutes of washing, after which further washing produced no change.

TABLE 2.
Weights of One Sample of Flesh Tissue after Various Periods of Washing.

Period of Washing.	Fresh Wt. (Observed.)	Dry Wt. (Calculated.)
0	4·00 gm.	0·58 gm.
5 min.	3·45 ,,	0·39 ,,
20 ,,	2·94 ,,	0·27 ,,
60 ,,	2·49 ,,	0·19 ,,
120 ,,	2·14 ,,	0·14 ,,
16 hr.	1·90 ,,	—

Respiration Rates of Tissue Sets washed in Aerated Distilled Water for Various Periods.—Two experiments were carried out to determine the respiration rates of comparable sets of mature apple discs which had been washed in aerated distilled water for various periods. Table 3 shows the results of one of these experiments.

TABLE 3.
Respiration Rate of Flesh of Mature Apple after Various Periods of Washing (25–26.ix.44). Material Weighed after Washing, before being Set Up in Warburg Vessels.

Period of Washing.	Respiration Rate (mm.³/gm. initial wt./hr.).		
	Replicates.		
	A.	B.	C.
0	16·5	16·0	15·5
5 min.	8·0	—	—
15–20 ,,	6·0	—	—
120 ,,	5·0	—	—
4½ hr.	4·0	—	—
19 ,,	5·0	6·0	—
25 ,,	5·5	4·0	—

Discs which were placed in the Warburg vessels with no preliminary washing settled down to relatively high respiration rates; discs which had been washed attained lower respiration rates than those which had not been washed; increasing the washing period beyond 15–20 minutes did not appear to decrease the respiration rate further. The results of the above experiments led to the adoption of a standard 20 minutes washing period for tissue used in the earlier investigations.

It is highly probable that the decrease in respiration rate of flesh with increased time of washing in distilled water (see Table 3) was due in some measure to the extensive loss of water and dry material which took place during the first 20 minutes of washing. In samples which had been washed for very short periods, leaching of solutes from the cells was still proceeding at a relatively high rate when the discs were transferred to the Warburg vessels (cf., Table 2), whereas in samples which had been washed for longer periods leaching was slow. In the former samples the amounts of solutes leached from the cells might have been sufficient to alter the concentration of the external solution in the Warburg vessels to such an extent as to prevent further leaching; this might have resulted in the maintenance of the respiration rates of these samples at relatively high levels. The fact that washing for more than 20 minutes did not bring about a further decrease in the respiration rate was probably due to the comparative smallness of the change in the dry wt./fresh wt. ratio which occurred after 20 minutes (see Table 2). No significant difference was observed in the respiration rates of tissue sets washed for various periods (from 5 min. to 5 hr.) in calcium chloride solution isotonic with the cell sap (see following section).

Effects of Immersion of Flesh in Solutions isotonic with Cell Sap.—When the effects of contact of flesh cells with distilled water were observed microscopically, it was found that many of the cells absorbed water so rapidly that disorganization of the contents occurred. In view of this, several experiments were carried out to determine the effects of immersion of discs of flesh in solutions of glucose, potassium nitrate and calcium chloride at various concentrations. It was found that loss in fresh weight after 2 hours immersion was much less in low concentrations of these substances than in distilled water. When the osmotic pressure of the surrounding solution was between 11 and 15 atmospheres the loss in fresh weight was very small or negligible after 2 hours. Losses in percentage dry wt. per unit fresh wt. were very small or negligible after various periods of washing, from 5 min. to 2 hr., in 0·25M calcium chloride (O.P. = 12·3 at.). The suction pressure of the flesh cells was between 11 and 15 atmospheres. When the osmotic pressure of the surrounding solution was greater than 15 atmospheres, the tissue lost weight owing to plasmolysis; when the osmotic pressure of the surrounding solution was less than 11 atmospheres the flesh tissue lost weight owing to rupture of the cells following rapid absorption of water from the surrounding solution. The suction pressure of the skin cells was of the same order as that of the flesh cells. When the osmotic pressure of the surrounding solution was lower than 11 atmospheres, the skin tissue gained in weight owing to absorption of water; the cells of the skin were not disorganized by immersion in solutions of low osmotic pressure.

Several experiments were carried out comparing the respiration rates of samples of flesh (unrinsed) in distilled water with those of similar samples in 0·25M calcium chloride. It was found that the shape of the respiration-time curve of tissue in calcium chloride was similar to that of tissue in distilled water, except that the initial fall was not as steep in calcium chloride as in water; the final respiration rate per unit initial weight was almost twice as high in calcium chloride as in water. The tissue sets were weighed at the close of the experiments. When the respiration rates were calculated per unit final weight it was found that the respiration rate of tissue in calcium chloride was only 15–20% higher than that of tissue in water.

There was no significant difference between the respiration rate of skin in 0·25M calcium chloride and that of skin in water.

In view of the fact that the respiration rate per unit true weight of flesh in water was so close to that of flesh in calcium chloride, it seemed that the main effect of immersion in the salt solution was the prevention of the occurrence of changes in weight during the experiments. In some tissues (e.g., carrot discs, Robertson, 1941) addition of chloride to the external medium results in chloride accumulation and increased respiration. This effect does not appear to be of major importance in apple flesh tissue, and there is complete absence of 'salt effect' in the skin.

In spite of its effects on weight loss, substitution of calcium chloride for water during washing did not greatly prolong the life of the cut flesh. The respiration rate of discs washed overnight in calcium chloride was no greater than that of discs washed overnight in distilled water.

The steepness of the initial fall in the respiration-time curve for flesh tissue in water was probably due in part to the loss in true weight which occurred after transference to the Warburg vessel. Respiration rate estimated on the basis of initial fresh weight would clearly have been erroneous, since fresh weight continued to decrease in the Warburg vessels. Making allowance for this decrease in weight and assuming that a proportionate number of cells ceased to respire, the respiration-time curve still showed a slight fall during the first 2 hours after setting up (see Figure 1). This fall might have been partly due to some of the cells ceasing to respire, as a result of disorganization, but not immediately losing their contents, and therefore contributing a small part of the weight of the tissue. The fact that there was still a slight initial fall in respiration rate when the discs were immersed in calcium chloride instead of in water indicated that some factor other than rapid disorganization of the cells was involved in the determination of the shape of the respiration-time curve.

Experiments with Tissue Slices of Different Thicknesses.—It was considered that if any fraction of the observed respiration rate of the flesh tissue had been due to the presence of cut cells, this fraction would have been increased in proportion to the increase of cut cells relative to uncut cells in a given weight of tissue. Several experiments were therefore carried out using tissue slices of various thicknesses. If an increase in the percentage of cut cells had accelerated the respiration rate (cf., work on other tissues) it might have been expected that this rate would have been highest in the thinnest discs. It was found, however, that whether the medium was water or calcium chloride, there was no significant difference in rate of oxygen uptake per unit fresh weight between tissue sets composed of discs 2 mm. thick and those composed of discs 1 mm. thick, and that in water, the rate of oxygen uptake of discs 0·5 mm. thick was slightly lower than that of discs 1 mm. thick. This difference could be largely accounted for if the damaged cells had lost most of their contents during washing and were therefore not contributing to the respiration. Table 4 shows the results of a typical experiment with discs of different thicknesses. From these data it is probable that no significant percentage of the observed final respiration rate of the tissue was contributed by cut cells.

TABLE 4.

Respiration Rates of Flesh Discs of Various Thicknesses. The Discs in Calcium Chloride were not from the Same Apple as Those in Water.

Thickness of Discs (mm.).	Respiration Rate (mm.^{3}/gm. fr. wt./hr.).	
	In Water.	In Calcium Chloride.
2·0	12·0	10·5
1·0	11·5	9·0
0·5	8·0	8·9

The results presented in the following pages for tissue in water have been corrected to the true weight of the tissue three hours after immersion (i.e., when most of the loss in weight had taken place).

RESPIRATION OF TISSUES FROM DIFFERENT REGIONS OF THE APPLE.

Respiration of Tissue from Different Depths in the Flesh.—In order to discover whether there was a gradient in respiration rate between the skin, the cells immediately under the skin and the cells in the deeper parts of the flesh, the rate of oxygen uptake was determined under similar conditions for each of the following regions of the same apple:

(1) skin;
(2) flesh tissue immediately under the skin, to a depth of approximately 3 mm.;
(3) tissue from 4 to 8 mm. under skin;
(4) tissue from 8 to 14 mm. under skin;
(5) flesh tissue more than 14 mm. under skin.

There was a very marked difference between the respiration rates of skin and flesh tissue; there was no significant difference between the respiration rates of flesh tissue from any of the regions considered.

In a second series of experiments the respiration rate of flesh tissue containing vascular strands was compared with that of flesh tissue containing no vascular strands. There was no significant difference in respiration rate between these two types of flesh tissue.

Respiration of Seeds.—The weight of seeds per fruit was no greater than 0·6 gm. It was observed that the respiration rate in air of seeds freshly removed from the fruit was of the same order per unit fresh weight as that of the flesh, or less. As the seeds constitute such a small percentage of the total weight of the fruit, their contribution to the total respiration of an uncut apple is very small.

Respiration of Skin and Flesh.—The respiration rate of the skin (expressed in mm.^{3}/gm. fr. wt./hr.) was found to be at least ten times as great (frequently 20 times as great) as that of flesh from the same apple expressed in the same units. This was true for Delicious and Jonathan apples as well as for Granny Smiths. When the respiration rates were expressed in mm.^{3}/gm. dry wt./hr., that of the skin was 4–5 times as great as that of the flesh. Table 5 gives data for two typical immature fruits. For Granny Smiths the ratio skin respiration/flesh respiration varied between approximately the same limits in mature fruits as in immature fruits.

TABLE 5.

Respiration Rates of Skin and Flesh in Immature Granny Smith Apples.

Date of Picking.	Tissue.	Oxygen Uptake. (mm.^{3}/gm. fresh wt./hr.). Value based on Weight at Time of Setting Up.	Oxygen Uptake. (mm.^{3}/gm. fresh wt./hr.). Corrected to Approx. Final Wt.	Oxygen Uptake. (mm.^{3}/gm. dry wt./hr.)
5. ii.45	Flesh	8·2	10·3	117
	Skin	150·0	—	625
19.iii.45	Flesh	8·0	10·0	145
	Skin	140·0	—	635

From results of the type shown in Table 5, it has been calculated that the skin contributes a considerable proportion of the total respiration of the apple. In a typical fruit, removed from store on 10.vii.45, the respiration of the skin was 150 mm.^{3}/gm. fr. wt./hr. and that of the flesh was 15 mm.^{3}/gm. fr. wt./hr. The fruit weighed 140 gm. fr. wt.; the skin constituted 4·3 gm., the remaining 135·7 gm. being composed of flesh, seeds, etc. Thus the total respiration of the skin was 645 mm.^{3}/fruit/hr., and the total respiration of the flesh was 2,040 mm.^{3}/fruit/hr. The total respiration of the skin plus flesh was therefore 2,685 mm.^{3}/fruit/hr. In this fruit, the skin was apparently responsible for a little less than one-third of the total respiration. The proportion of the total respiration contributed by the skin varied in individual fruits. It was nearly always of the order of one-third, but in rare cases values as high as one-half were recorded.

The Relation between Skin Respiration and Flesh Respiration throughout Post-Storage Life.—The magnitudes of the respiration rates of skin and flesh varied among individual fruits. It has been shown (Hackney, 1943) that the respiration rate of the whole fruit decreases with time after removal from store. The data obtained during 1944 and 1945 show that this decrease in total respiration is probably made up of decreases in both the respiration of the skin and that of the flesh; the ratio skin respiration/flesh respiration probably remains constant throughout the post-storage life of each individual fruit.

In 1944 a strong positive correlation was observed between the respiration rates in water of skin and flesh from the same fruit. The regression line was $Y = 6{\cdot}136X + 49{\cdot}58$, where Y = respiration of skin and X = respiration of flesh, and the regression coefficient was highly significant ($P < 0{\cdot}001$). A similar correlation was indicated in 1945, but the data were not numerous enough to merit statistical analysis.

The Anatomy of the Skin and Flesh.—The cells of the skin are much smaller and more tightly packed than those of the flesh. The percentage dry weight per unit fresh weight is 4–5 times as great for skin as for flesh. Figures 2 and 3 show the cellular detail of mature apple tissue. Immediately below the cuticle is a layer of irregular polygonal cells of small diameter (about 20μ), each containing a comparatively small vacuole and dense cytoplasm, in which a large number of chloroplasts is visible. Figure 2 shows a surface view of the epidermal layer. In cross-section (Figure 3) it appears to be about as thick as the cuticle. Immediately under the epidermis are about three layers of flattened cells about twice as large in diameter as the epidermal cells, but slightly smaller in radial thickness. These cells contain relatively dense cytoplasm with many chloroplasts. The 3–4 layers immediately under this subepidermal region show a gradual transition from the small, densely packed, chlorophyllous cells of the subepidermis to the large, loosely packed, non-chlorophyllous cortical cells of which the greater part of the apple is composed. The tissue used as 'skin' in the experiments described here includes only the first 7–8 layers of cells beneath the cuticle.

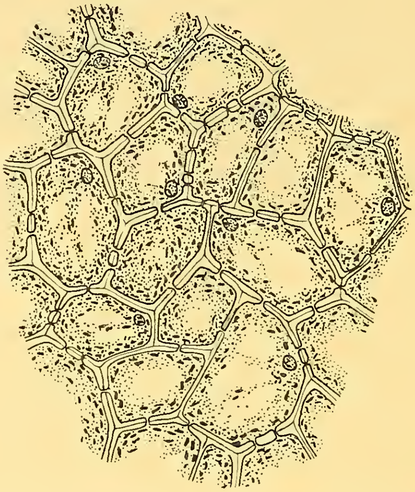

Fig. 2.—Surface view of the epidermal cells of the Granny Smith apple, showing dense cytoplasmic contents with many chloroplasts (× 500).

The flesh tissue is composed of very loosely arranged cells, having between 10 and 20 times the volume of the cells comprising the skin. Each of these cells contains a thin layer of cytoplasm and a large vacuole. The intercellular spaces are large.

The large volume of the individual cells and the relative thinness of their cytoplasmic layers are probably the cause of the disorganization which occurs on immersion of the flesh in distilled water or in solutions of low osmotic pressure. The cytoplasmic layers of some of the cells are probably ruptured by the sudden expansion of the very large vacuoles consequent upon the absorption of water from the external solution.

The cellular details of Jonathan and Delicious apples were found to be very similar to those of the Granny Smith. The cuticle of the Delicious apple is thinner than those of the Granny Smith and Jonathan. Tetley (1930, 1931) described the anatomy of several varieties of apple grown in England. The Granny Smith appears to be more similar to the Bramley's Seedling than to the other varieties examined by Tetley, but there are differences in anatomical detail between the two varieties; the epidermal cells of the Granny Smith are much smaller than those of the Bramley's Seedling (Tetley, 1931).

From a microscopic study of the structure of the skin and flesh, it is clear that the flesh contains a large proportion of vacuolar material, which is not actively concerned in respiration, and a small proportion of cytoplasm, whereas the skin contains a small proportion of vacuolar material and a large proportion of actively respiring cytoplasmic material. A rough estimation was made of the numbers of cells in 1 gramme of flesh and skin:

Volume of 1 gm. flesh = 11×10^2 mm.^{3} (approx.).
Volume of 1 gm. skin = 9×10^2 mm.^{3} (approx.).
Volume of a flesh cell was of the order of 2×10^{-4} mm.^{3}.
Therefore the number of cells in 1 gm. flesh was of the order of $5{\cdot}5 \times 10^6$.

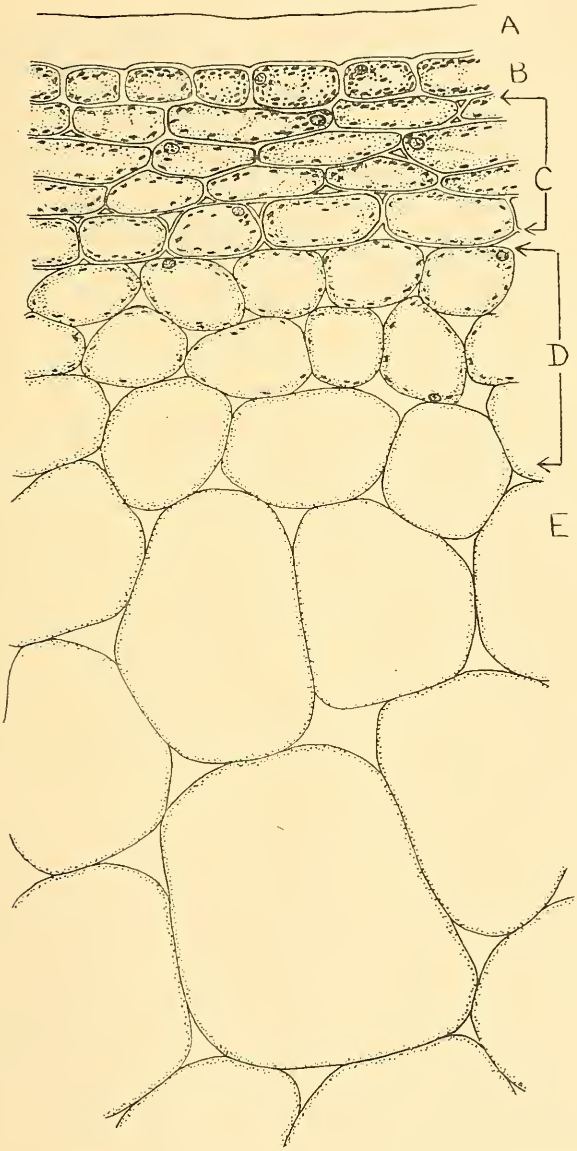

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Fig. 3.—Transverse section of skin and flesh of Granny Smith apple. A, Cuticle; B, Epidermis; C, Subepidermis; D, Transition zone; E, Flesh (×500).

The skin was made up of cuticle plus epidermis plus subepidermis plus transition tissue. From sections of the type shown in Figure 3 the following calculations were made:

Volume of cuticle in 1 gm. skin = 100 mm.^{3} (approx.).
Volume of epidermal tissue in 1 gm. skin = 66 mm.^{3} (approx.).
Volume of subepidermal tissue in 1 gm. skin = 300 mm.^{3} (approx.).
Volume of transition tissue in 1 gm. skin = 450 mm.^{3} approx.).
Average volume of epidermal cell = $1{\cdot}8 \times 10^{-6}$ mm.^{3} (approx.).
Therefore number of epidermal cells in 1 gm. skin = $(66/1{\cdot}8) \times 10^6$ (approx.).
Average volume of a subepidermal cell = $3{\cdot}3 \times 10^{-6}$ mm.^{3} (approx.).
Therefore number of subepidermal cells in 1 gm. skin = $(300/3{\cdot}3) \times 10^6$ (approx.).
Average volume of transition cells = 36×10^{-6} mm.^{3} (approx.).
Therefore number of transition cells in 1 gm. skin = $(450/36) \times 10^6$ (approx.).
Thus total number of cells in 1 gm. skin was of the order of 140×10^6.

The ratio (number of cells in 1 gm. skin)/(number of cells in 1 gm. flesh) was 25/1 approximately. As this was of the same order as the ratio (respiration of 1 gm. skin/hr.)/(respiration of 1 gm. flesh/hr.), the difference in magnitude between skin respiration and flesh respiration might be accounted for by the greater number of cells per unit fresh weight in the skin than in the flesh; the average respiration rate per cell is approximately the same in different parts of the fruit. This is analogous to the constancy of the respiration rate per cell previously shown in developing Granny Smith apples (Hackney, 1944).

Correlation between Respiration Rate of Flesh Tissue and Respiration Rate of the Whole Apple before Cutting.

During August, 1944, a series of experiments was carried out in which the respiration rate of flesh tissue was compared with the respiration rate of the whole apple prior to cutting. Three samples of 25 apples each were removed from store on 1.viii.44, 14.viii.44 and 28.viii.44 respectively. Using the Pettenkofer technique, the rate of carbon dioxide output (mg./10 Kg./hr.) was determined for each apple during the 48 hours immediately before cutting. Some of the apples were cut within two days after removal from store; others had been out of store for as long as 1½ weeks before being cut. The respiration rate of the whole fruit was falling steadily during the post-storage life (cf., Hackney, 1943). The rate of oxygen uptake in distilled water of a 2 gm. sample of flesh tissue from each apple was then determined. Assuming that the respiratory quotient was approximately 1 (Hackney, 1944), the values for oxygen uptake of flesh were converted from mm.^{3}/gm./hr. to their equivalents in mg. CO_2/10 Kg./hr., for purposes of comparison with the respiration rates of the uncut apples. Table 6 shows a typical set of results, given by the first sample of apples. Each pair of figures refers to a single apple.

When the data from all three batches were considered, it was found that there was a strong positive correlation between the respiration rate of the whole fruit and that of flesh tissue cut from it. The regression coefficient was 0·75 and was highly significant ($P << 0{\cdot}001$). A similar correlation was indicated during 1945, but the data were not numerous enough to merit statistical analysis. It appears that when the respiration of the whole apple is high, that of its flesh tissue is high; when the respiration of the whole apple is low, owing to length of time after removal from store or to individual variability, the respiration of its flesh tissue is also low.

The respiration rate of skin was not determined in this experiment. However, in view of the correlation observed in other experiments between the respiration rates of skin and flesh (see earlier discussion), it is highly probable that the respiration rate of the skin was correlated with that of the whole fruit.

COMPARISON BETWEEN RESPIRATION RATE OF WHOLE APPLE AS DETERMINED DIRECTLY BY THE PETTENKOFER METHOD AND AS CALCULATED INDIRECTLY BY THE WARBURG METHOD.

In order to compare the respiration rate of the whole apple after cutting with that of the whole apple prior to cutting, it was necessary to determine the respiration rate of the cut skin as well as that of the flesh.

TABLE 6.

Comparison between Respiration Rate of Whole Apple and that of Flesh Tissue.

Respiration of Whole Apple at 21° C. (mg./10 Kg./hr.)	Respiration of Flesh Tissue at 21° C. (mg./10 Kg./hr.) (Calc. on True Fresh Wt. at Close of Exp.
270	373
250	307
225	293
217	346
210	266
210	293
207	266
205	293
200	260
195	266
200	277
195	293
195	266
190	213
160	173
140	227
140	253

On 30.x.45 two apples were removed from store and held at 25°C. The concentrations of oxygen and carbon dioxide in their internal atmospheres were determined by the method described by Trout *et al.* (1942). It was found that the fruits were probably at the stage where internal oxygen concentration was limiting respiration rate (cf., Hackney, 1943). The respiratory drifts of the two fruits were observed, using the Pettenkofer method. All the skin was then removed from the fruits and weighed; the remainders of the total weights of the fruits were regarded as 'flesh'. The respiration rates of samples of skin and flesh in 0·25M calcium chloride were then determined, using the Warburg method. From the respiration rates of these samples it was possible to calculate the respiration rate of the whole fruit. The respiratory quotient of an intact apple (Hackney, 1944) and of cut apple tissue under the conditions of the experiment were both approximately equal to unity, i.e., the volume of carbon dioxide given off was approximately equal to the volume of oxygen taken up. Both fruits behaved similarly. The following data were obtained with one of them:

Respiration rate of flesh = 7·3 mm.^{3}/gm. fr. wt./hr.
= (7·3/500) mg./gm./hr.
Total weight of flesh = 125 gm.
Therefore total respiration of flesh = 1·83 mg./fruit/hr.
Respiration rate of skin = 135 mm.^{3}/gm. fr. wt./hr.
= (135/500) mg./gm./hr.
Total weight of skin = 4·34 gm.
Therefore total respiration of skin = 1·17 mg./fruit/hr.
Therefore calculated respiration of whole fruit = 3·0 mg./fruit/hr.
= 233 mg./10 Kg./hr.

The observed respiration rate of this fruit prior to cutting was 200 mg./10 Kg./hr. Thus the calculated respiration rate was 33 units higher than the observed rate. As mentioned previously, the apples were probably at the stage where internal oxygen supply was limiting respiration rate. The internal oxygen concentration was governed by the resistance of the skin to the passage of oxygen (Hackney, 1943). When the tissues were cut and exposed to the air, the oxygen supply was suddenly increased to approximately 21% (the internal oxygen concentrations of the two fruits prior to cutting were 7·0% and 6·2% respectively). In order to determine whether the difference between observed respiration rate prior to cutting and calculated respiration rate after cutting could be attributed to the change in oxygen supply to the tissues, the skin and flesh were surrounded with gas mixtures containing higher percentages of nitrogen and lower percentages of oxygen than are normally present in air. Table 7 shows the calculated respiration rates of the two fruits in these gas mixtures.

TABLE 7.

Comparison of Respiration Rates of Whole Fruits (mg./10 Kg./hr.) obtained by Pettenkofer and Warburg Methods; Internal Oxygen Concentrations prior to Cutting; Effects on Calculated Respiration of Lowering Oxygen Supply.

Fruit No.	Int. O_2 (%).	Resp'n. (Pettenkofer).	Resp'n. (Warburg).		
			In Air.	In 11% O_2.	In 6% O_2.
1	7·0	200	233	223	190
2	6·2	196	221	215	190

It is clear from the above table that, when the cut tissues were surrounded with a gas mixture containing a percentage of oxygen approximately equal to that present in the internal atmosphere prior to cutting, the calculated respiration rate of the whole fruit was very close to the observed value prior to cutting. This indicates (1) absence of prolonged stimulation of respiration due to cutting, and (2) pronounced effect of increase in oxygen concentration within certain limits on the respiration rate. The second point is in accordance with the results obtained in previous years when whole apples were held in an atmosphere of pure oxygen (Hackney, 1944).

When the skin and flesh were returned to air after being surrounded with the 6% oxygen mixture the calculated respiration rate of the whole fruit returned to the air level. Substitution of 100% oxygen for air did not result in any further increase in respiration rate.

The change brought about in respiration by decreasing the oxygen supply from 11% to 6% was greater than that brought about by decreasing it from 21% to 11%. In the two fruits used, the effects of changes in oxygen concentration were apparently greater at lower oxygen concentrations than at higher oxygen concentrations. Another point of interest is that these effects were much more pronounced on skin tissue than on flesh tissue. This may have been due to the respiration rate per unit weight being so much higher in the skin than in the flesh.

The experiment described above indicated the possibility of using the cut tissue technique as a means of investigating the respiration of apple tissue under conditions where oxygen supply is not limited by the presence of the skin.

SUMMARY.

The technique for the determination of the respiration rate of sliced apple tissue has been described and discussed in detail.

Respiration rates were determined for tissues from different regions of the Granny Smith apple. There was no significant difference in respiration rate between flesh tissue slices from various parts of the apple, but the respiration rate of the skin per unit weight was many times higher than that of the flesh. This was shown to have been probably due to the greater number of cells per unit weight in the skin than in the flesh.

There was a strong positive correlation between the respiration rate of the skin and that of the flesh from the same apple.

There was a strong positive correlation between the respiration rate of the whole apple and that of flesh tissue cut from it.

Values for the total respiration rates of whole apples, calculated from the respiration rates of skin and flesh tissue, were greater than the values obtained directly with the uncut apples. This increase in total respiration rate after cutting was shown to have been probably due to the increasing of the oxygen supply to the tissues consequent upon cutting.

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